

WHAT IS CLAIMED IS:

1. A high throughput and quantitative method of analyzing post-translational protein modifications in a sample 5 comprising the steps of:

a) preparing at least one of N identical arrays of immobilized protein capture agents, each of said capture agents binding specifically to a protein in said sample; and

b) performing in any order the steps comprising:

10 i) applying said proteins of the sample to at least one of said N arrays of immobilized protein capture agents; and

15 ii) binding said proteins of the sample to at least one of X detectable affinity reagents to label said proteins, wherein X is an integer from 1 to N and wherein each of said X detectable affinity reagents specifically recognizes one of N post-translational protein modifications; and

20 c) measuring a signal "associated" with said detectable affinity reagents, wherein quantitation of said signal from said X detectable affinity reagent(s) provides a high throughput and quantitative analysis of post-translational protein modifications in said sample.

25 2. The high throughput and quantitative method of claim 1, wherein step b comprises the order of:

binding said proteins of the sample to X detectable affinity reagents to label said proteins;

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applying said labeled proteins to at least one of said N arrays of immobilized protein capture agents; and

binding said labeled proteins captured in at least one of said N arrays to (N-X) of said detectable affinity reagents.

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3. The high throughput and quantitative method of claim 1, wherein step b comprises the order of:

binding said proteins of the sample to all of said X detectable affinity reagents to label said proteins; and

applying said labeled proteins to at least one of said N arrays of immobilized protein capture agents.

15 4. The high throughput and quantitative method of claim 1, wherein step b comprises the order of:

applying said proteins of the sample to at least one of said N arrays of immobilized protein capture agents; and

20 binding said captured proteins to all of X detectable affinity reagents.

25 5. The method of claim 1, wherein each of X affinity reagents are detectably distinct from said first affinity reagent and from each other.

6. The method of claim 1, wherein if one of each of a second through N affinity reagents is applied separately to said labeled proteins captured in one each of said identical N arrays, said affinity reagents are detectably identical.

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7. The method of claim 1, wherein said protein capture agents are antibodies, antibody fragments, recombinant proteins, nucleic acids, or phage particles.

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8. The method of claim 1, wherein said affinity reagents are antibodies, antibody fragments, recombinant proteins, nucleic acids, or phage particles.

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9. The method of claim 8, wherein said affinity reagents are directly labeled with a detectable tag.

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10. The method of claim 8, wherein said affinity reagents are labeled with a secondary detectable affinity reagent.

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11. The method of claim 10, wherein said secondary affinity reagent is an antibody, an antibody fragment, a recombinant protein, a nucleic acid or phage particle.

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12. A high throughput and quantitative method of comparative analysis of post-translational protein modifications in different samples, comprising the steps of:

10 preparing an array of immobilized protein capture agents, each of said capture agents binding specifically to a protein in said samples;

15 incubating a first sample A with an affinity reagent, said affinity reagent labeled with a first detectable label, wherein said affinity reagent specifically recognizes a post-translational protein modification;

incubating a second sample B with said affinity reagent, said affinity reagent labeled with a second detectable label;

20 applying a mixture of said affinity reagent-labeled samples A and B to said array of immobilized protein capture agents;

25 quantifying relative signals from said first and said second detectable labels on said affinity reagents; and

calculating the ratios of said relative signals from said first and said second detectable labels, wherein said ratios correlate to the relative abundance of said post-translational modifications between said sample A and said sample B.

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13. The method of claim 12, wherein said protein capture agents are antibodies, antibody fragments, recombinant proteins, nucleic acids or phage particles.

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14. The method of claim 12, wherein said affinity reagent is an antibody, an antibody fragment, a recombinant protein, a nucleic acid, or a phage particle.

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15. The method of claim 12, wherein said detectable labels are fluorophores, nucleic acids or enzymes.

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16. A high throughput and quantitative method of comparative analysis of post-translational protein modifications in different samples, comprising the steps of:

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preparing an array of immobilized protein capture agents, each of said capture agents binding specifically to a protein in said samples;

incubating a first sample A with an affinity reagent, said affinity reagent labeled with a first fluorophore, wherein said affinity reagent specifically recognizes a post-translational protein modification;

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incubating a second sample B with said affinity reagent, said affinity reagent labeled with a second fluorophore;

applying a mixture of said affinity reagent-labeled samples A and B to said array of immobilized protein capture agents;

5 measuring the fluorescence emission of said first and said second fluorophores, and

calculating the ratios of relative fluorescence of said first and said second fluorophores, wherein said ratios correlate to the relative abundance of said post-translational modifications between said sample A and said sample B.

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17. The method of claim 16, wherein said protein capture agents are antibodies, antibody fragments, recombinant proteins, nucleic acids or phage particles.

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18. The method of claim 16, wherein said affinity reagent is an antibody, an antibody fragment, a recombinant protein, a nucleic acid, or a phage particle.

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19. A high throughput and quantitative method of analyzing protein interactions, comprising the steps of:

25 preparing an array of immobilized protein capture agents, each of said capture agents binding specifically to a protein in said sample;

labeling the proteins in said sample with a first fluorophore;

applying the labeled proteins to said array of immobilized protein capture agents;

5 labeling molecules with a second fluorophore;

applying said labeled molecules to the labeled proteins captured on said array of immobilized capture agents, said molecules specifically binding to the labeled proteins captured on said array of immobilized capture agents; and

10 measuring the emission of said first and said second fluorophores, wherein the relative fluorescence of said first and of said second fluorophores correlates with an interaction of said molecules with the proteins thereby providing high throughput and quantitative analysis of the protein interactions.

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20. The method of claim 19, wherein said protein capture agents are antibodies, antibody fragments, recombinant proteins, nucleic acids, or phage particles.

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21. The method of claim 19, wherein said molecules are selected from the group consisting of protein molecules, small molecules, drug molecules and nucleic acid molecules.

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22. A kit for a high throughput and quantitative method of analyzing post-translational protein modifications comprising:

at least one array of immobilized protein capture
5 agents;

at least one buffer medium;

at least one affinity reagent, each of said affinity reagents recognizing a specific post-translational protein modification.

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23. The kit of claim 22, wherein said protein capture agent(s) is an antibody, an antibody fragment, a recombinant protein, a nucleic acid or phage particles.

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24. The kit of claim 22, wherein said affinity reagent(s) is an antibody, an antibody fragment, a recombinant protein, a nucleic acid or phage particles.

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25. A kit for a high throughput and quantitative method of analyzing post-translational protein modifications comprising:

25 at least one array of immobilized protein capture agents; and

at least one buffer medium.

26. The kit of claim 25, wherein said protein capture agent(s) is an antibody, an antibody fragment, a recombinant protein, a nucleic acid or phage particles.

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27. A kit for a high throughput and quantitative method of analyzing post-translational protein modifications comprising:

10 at least one affinity reagent, each of said affinity reagents recognizing a specific post-translational protein modification; and

at least one buffer medium.

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28. The kit of claim 27, wherein said affinity reagent(s) is an antibody, an antibody fragment, a recombinant protein, a nucleic acid or phage particles.

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29. A kit for a high throughput and quantitative method of analyzing post-translational protein modifications comprising:

25 at least one array of immobilized protein capture agents; and

at least one affinity reagent, each of said affinity reagents recognizing a specific post-translational protein modification.

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30. The kit of claim 29, wherein said protein capture agent(s) is an antibody, an antibody fragment, a recombinant protein, a nucleic acid or phage particles.

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31. The kit of claim 29, wherein said affinity reagent(s) is an antibody, an antibody fragment, a recombinant protein, a nucleic acid or phage particles.

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32. A kit for a high throughput and quantitative method of analyzing post-translational protein modifications comprising:

a set of buffer media.